

Original article

Compression garments to prevent delayed onset muscle soreness in soccer players

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The purpose of this study was to evaluate the use of a compression garment as DOMS prevention. This was accomplished by provoking a DOMS in 15 athletes, running on a treadmill at 73% of their maximal aerobic velocity, during 40 minutes with a 10% negative slope; wearing the compression garments on one thigh, protected thigh (PT), and not in the contralateral thigh, control thigh (CT).

A clinical and MRI diagnosis of DOMS was performed. Biopsies in both vastus lateralis were done, and the amount and severity of the DOMS was estimated by measuring intracellular albumin, and lymphocytes CD3+ and neutrophils intra/interfibrillar infiltrates, 48h after the induced damaging exercise.

There was less total injury in the PT than in the CT, a 26.7% average. These data indicate that this compression garment is an effective method to reduce the histological injury in DOMS.

KEY WORDS: albumin, biopsy, eccentric, inflammatory cells, muscle.

Introduction

Delayed onset muscle soreness (DOMS)¹ is the discomfort and pain while the muscle contracts, it appears after doing unusual exercise, and normally although not exclusively, related to eccentric muscular work^{2,3}. Muscle soreness is accompanied by a feeling of stiffness as a result of muscle oedema⁴, as well as a loss of strength and range of movement (ROM)^{5,6}. The signs and symptoms begin between 6-12 h after exercise, increase progressively until reaching a peak pain between 48-72 h, and reduce until they disappear between 5-7 days later^{2,7,8}. DOMS is also accompanied by biochemical alterations like an increase of CPK in the blood⁹. In some studies it is classified as a muscle injury grade 1¹⁰, although its clinical evolution is different^{1,11}. In fact, it has been used as an experimental model to study the muscle injury¹².

Compression garments as a therapeutic or preventative measure, are normally used in different scopes of medicine (vascular pathology, plastic surgery, in sports medicine). Multiple articles have appraised the effect of the compressive garments on muscle function¹²⁻²³ recovery post exercise²⁴⁻²⁹, motor control³⁰, thermoregulation³¹, warming up¹⁷ and ROM^{17,32}. Compression garments for DOMS recovery have also been studied by applying compression measures after the bout of exercise^{27,33-36}, however, the compression garments assessed are made of distinct materials and they differ in composition, architecture or degree of compression, making difficult to compare results. Moreover, compression garments have not been studied as a preventative strategy during provocation.

Signs of histological muscle damage in DOMS are myofibrillar structure disruptions and myofibre necrosis and inflammation³⁷. Sarcolemmal membrane injury allows extracellular proteins like albumin to be detected intracellularly, this is a sign of membrane injury^{38,39}.

The inflammatory reaction within myofibres is a sign of segmental myofibre degradation and necrosis, neutrophils (MPO) and lymphocytes CD3+ (CD3+) reflect the amount and intensity of cellular damage^{37,40}.

Thus, the aim of this study is to evaluate if there is a protective effect of compression against DOMS; more specifically, the protective effect of this compression garments against DOMS. For this purpose we will evaluate the amount of damaged fibers and the severity of this damage by measuring intracellular albumin, lymphocytes CD3+ (CD3+) and neutrophils

(MPO) intra/interfibrillar infiltrates, after the induced damaging exercise.

Methods

Experimental approach to the problem

An experimental, prospective study, involving healthy athletes with no clinical history of interest, and who regularly did physical activity, was designed. Each individual served as own control, since one thigh wore the compression garment, protected thigh (PT), while the other remained unprotected, control thigh (CT), Figure 1.



Figure 1. Right thigh protected and left without compression garment.

Subjects

Adult male amateur soccer players from two different local clubs, older than 18 years, with a regular training activity from 4-12 hours per week during at least 3 months, were considered to take part in the study. Three weeks before the study they were instructed to eat normally, and to avoid: unusual physical activity, alcohol, and any toxic substances until the end of the study. Neither did they have any medical or pharmacological (NSAID, analgesic) treatment, or any physical measurement that could alter the result of the injury (cryotherapy, warm baths, massage, electrotherapy, etc.).

Previous to the inclusion, the subjects were informed about the protocol and its objectives and gave their consent to take part in the study. The project was previously assessed and approved by the ethics committee of the *Consell Català de l'Esport* (0099S/ 4882/2010).

Sixteen individuals were initially included in the study; nevertheless, the final sample was 15 because one of the subjects could not complete the study. The main physical and physiological baseline characteristics of the subjects evaluated are shown in Table 1. Additionally, the distribution of the subjects leg dominance, together with randomization for the compression, are shown in Table 2. Athletes were allocated in the right or left thigh compression group following a randomization process by taking random numbers from a com-

puter program, a summary of the protocol is shown in Figure 3A, and the flow Diagram Figure 3B.

Procedures

Compression tights: the compression tights used in the study (Colibri®, Puntiblon, Spain) are made of a combination of a compression fabric (polyamide 57% and elastomer 43%), with inner reinforcements over the quadriceps and hamstrings, Figure 2 A and B. The tights composition and its 3D structure allow it to control the elasticity at 360°, Figure 2-C⁴¹. The level of compression was not measured, all subjects used the smaller size garment they could wear, and the choice of size was performed under the supervision of the authors.

Submaximal test and bout of eccentric exercise: nine days before the DOMS provocation, each subject performed an incremental submaximal exercise test on a motorised treadmill (EG2, Vitoria, Spain), oxygen consumption was measured using a computerised metabolic cart (Master Screen CPX, Erich Jaeger, Wuerzburg, Germany). The test protocol start 3 minutes at 8 km/h with 3% of positive slope, and then 1 km/h speed increases every minute until to reach the 90% of the theoretical maximal heart rate. The test was used to determinate the subject's speed for the DOMS provocation exercise.

The bout of eccentric exercise started with 10 minutes running at free speed on a treadmill (EG2, Vito-

Table 1. Descriptive statistics, patient and test variables. MAP: Max. Aerobic Power, MAV: Max. Aerobic Velocity, MV: Max. Velocity, AnTV: Anaerobic Threshold Velocity. *: Median (Min-Max).

	Mean	
Age (years)	25.0	(19-50) *
Height (cm)	177.6	(3.3)
Weight (kg)	78.2	(5.8)
BMI (Kg/m ²)	24.7	(1.6)
MAP (ml)	3433	(594)
MAV (km/h)	13.9	(1.7)
VO ₂ mL/(kg*min)	44.0	(7.6)
MV (km/h)	13.8	(1.7)
AnTV (km/h)	11.2	(1.9)
VO ₂ Ua (ml)	2768	(588)
Heart Rate (bpm)	163	(11.7)
Test Velocity (km/h)	10.7	(1.7)

Table 2. Treatment randomization results, according to leg characteristics. R: Right, L: Left.

	Dominant Leg		Total
	R	L	
Compression R	6 (50%)	1 (33%)	7 (47%)
Compression L	6 (50%)	2 (67%)	8 (53%)
Total	12	3	15

ria, Spain), as a warm up, and without stop, 40 minutes with a 10% downhill slope, at 73% of the maxi-



Figure 2. Compression garment, A: external surface, B: internal surface, C: fabric architecture.

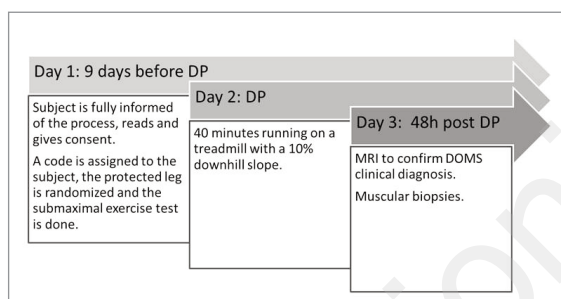


Figure 3A. Study protocol. DP: DOMS provocation.

mum speed reached in a submaximal test, done nine days before. Randomly, the subjects wore the compression garment on one thigh, the PT group, while the other thigh was cut and left unprotected, the CT group³².

DOMS Evaluation: an initial clinical diagnosis of DOMS, was confirmed by performing a magnetic resonance imaging (MRI)⁴². The degree of histological injury was assessed by taking a biopsy of the *vastus lateralis* of both thighs and measuring, intracellular albumin, and CD3+ and MPO intra/interfibrillar infiltrates, 48 h. after the bout of eccentric exercise.

MRI: a high magnetic field system was used (Signa 1.5 T G.E. Milwaukee). The patients were placed in the *supine decubitus* position and entered the exploration tunnel head first. Both thighs were explored with a coil body. The diagnosis was carried out looking for alterations in the MRI signal in any muscular group both in the flexor compartment and the extensor and its asymmetry as regards the contralateral homonymous muscular group. When the radiologist read the results he did not know which thigh was protected with the thigh being studied²².

Biopsies: the biopsies were obtained using a Vacora System Biopsy pistol (Bard), with a coaxial needle of 10G X140mm. With this technique the sample is aspirated and remains in the branula until it is extracted; the sample is taken out as a whole and not fragmented. Two biopsies were carried out from the middle third of each *vastus lateralis*, under ultrasound control. Previously, a cutaneous incision was performed with a 5 mm blade, the entrance was the same for both biopsies but the needle direction was modified^{6,22,42}. Once the samples were extracted, they were kept in formol, glutaraldehyde and a third sample was cryopreserved at -80°C for further studying⁴³.

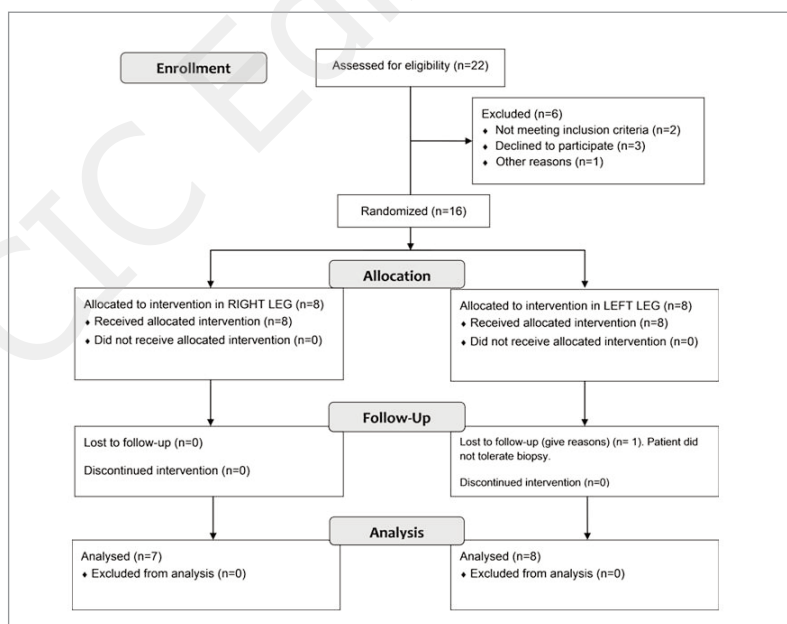


Figure 3B. Flow diagram.

Sarcolemmal disruptions evaluation: cellular membrane permeability to albumin is a sign of membrane injury³⁷. For histochemical procedures, all muscle specimens were first dissected free of visible connective tissue and fat and embedded in paraffin using conventional methods⁴⁶. Ten-micrometre sections were cut, varying the inclination of the holder by 5 degree increments until the minimum cross-sectional area was obtained, which was defined as truly transverse. For assessment and quantification of muscle membrane injury we chose a method based on light microscopy for identification of fibers that contain albumin by immunohistochemistry. Each sample was processed for immunohistochemical techniques using a polyclonal rabbit anti-human antibody directed against albumin (Code No. A0001; Dako Cytomation, DK-2600 Glostrup, Denmark) as a primary antibody. This immunocomplex was detected using a horseradish peroxidase-labeled goat anti-rabbit secondary antibody (Code No. K4003; EnVision+System-HRP labelled polymer, Dako Co., Carpinteria, CA, USA). The reaction was developed with a chromogen solution with 3,3-diaminobenzidine (Code No. K3468; Liquid DAB+ Substrate-Chromogen Solution, Dako Co., Carpinteria, CA, USA). The analysis of intracellular albumin was performed by two independent observers using a categorical scale (0-3) with a light microscope (Olympus, Series AX70TF; Olympus Optical Co., Shinjuku, Tokyo, Japan) coupled with an image-digitizing camera (View Finder Lite; Version 1.0.143c; Pixera Co., Los Gatos, CA) and a morphometry program (Scion Image, Version Beta 4.0.2; Scion Co., Frederick, MD, USA). Qualification of fiber injury was performed in a four-category finite interval system, the extremes representing either the absence of intracellular albumin (i.e., absence of sarcolemmal injury, degree 0), or presence of intracellular albumin on the complete cellular area (i.e., severe sarcolemmal injury, degree 3), Table 3. The two intermediate categories were determined by degree 1 injury (i.e., mild sarcolemmal injury or presence of albumin in less than 50% of the fiber area) and degree 2 injury (i.e., moderate sarcolemmal injury or presence of albumin on more than 50% of the fiber area, but not in all of it). Fiber categories were expressed as proportion (%) of total muscle fibers. The mean value of degree 2 and degree 3 obtained by two observers was used for statistical analysis^{44,45}.

Table 3. Fiber injury degrees description.

Degree	Description
0	Absence of intracellular albumin.
1	Presence of albumin in less than 50% of the fiber area.
2	Presence of albumin on more than 50% of the fiber area, but not in all of it.
3	Presence of intracellular albumin on the complete cellular area.

CD3+ and MPO Immunohistochemical staining: for assessment and quantification of CD3+ and MPO intra/interfibrillar infiltrates we chose a method based on light microscopy for identification of CD3+ and MPO by immunohistochemistry.

For the immunohistochemical assay we used manual immunostaining. For MPO with a polyclonal rabbit anti-human antibody directed against MPO (Dako Ref. IS511, 1:4 dilution, prediluted), and for CD3+ a polyclonal rabbit anti-human directed against CD3+ (Dako Ref. A0452, 1:250 dilution), used as a primary antibodies.

For MPO antigen retrieval the sections were deparaffinized, rehydrated in gradually decreasing concentrations of ethanol, PBS (3x5'), citrate 7,3 retrieval, pressure cooker, PBS (3x5'), hydrogen peroxide 10' at room temperature, PBS (3x5'), primary antibody 30' at room temperature 1:4 dilution, PBS (3x5'), secondary antibody Envision 30' at room temperature, PBS (3x5'), DAB (1 drop for 1 ml dilute) 5/10 minutes at room temperature, PBS (3x5'), Mayer haematoxylin 10' at room temperature, water, dehydration, D.P.X. assembly, visualization with Envision/HRP Dako (Glostrup, Denmark).

For CD3+ antigen retrieval the sections were deparaffinized, rehydrated in gradually decreasing concentrations of ethanol, PBS (3x5'), citrate 7,3 retrieval, pressure cooker, PBS (3x5'), hydrogen peroxide 10' at room temperature, PBS (3x5'), primary antibody 30' at room temperature 1:250 dilution, PBS (3x5'), secondary antibody Envision 30' at room temperature, PBS (3x5'), DAB (1 drop for 1 ml dilute) 5/10 minutes at room temperature, PBS (3x5'), Mayer haematoxylin 10' at room temperature, water, dehydration, D.P.X. assembly, visualization with Envision/HRP Dako (Glostrup, Denmark).

The lymphocytic infiltrates (CD3+) and MPO were quantified following the total number of T cells immunostained antibodies against CD3+, and MPO. The total number of CD3+ cells, and the total number of fibres were counted blindly by two observers, and was used for statistical analysis. CD3+ cells per fibre was calculated and compared between PT and CT⁴⁰. Number of fibres with MPO was evaluated in the same way^{47,48}.

The samples were blinded to the laboratory and the statistical analysis, coded with numbers and letters to identify patients and treatment allocation.

Statistical Methods

Initially a descriptive analysis was performed that includes: summary tables with mean, median and standard deviation were obtained for baseline variables (Age, Height, Weight, BMI, Max. Aerobic Power, VO2 ml/(kg* min), Max. Velocity, Anaerobic Threshold Velocity, VO2Ua, Max. Aerobic Velocity, Heart Rate and Test velocity).

Biopsy results, total injury, intracellular albumin, CD3+, MPO and CD3+, of each thigh (control vs. protected), were compared, bivariate analysis was done using the Student-Fisher t-test for paired data or the Wilcoxon

non-parametric test for non-normal distributions. The assumption of normality was verified using the Shapiro-Wilk test. Means of the differences with its 95% confidence intervals (IC95%) were calculated. The results of the subjects' thighs were graphically represented in a scatter plot for a better visualization of the data.

In order to analyse the variables of the biopsy adjusting for patient's characteristics and stress test generalised linear models⁴⁹ were used. A normal distribution was considered for Total injury, a Multinomial distribution for intracellular albumin, and a Poisson distribution for CD3 and MPO. Covariates were recorded in two categories using the median as cutting point. Covariates included in the final model were obtained using a backward stepwise selection method (variables that were not statistically significant were removed from the model).

The analysis was performed with commercially available software (SAS v9.2, SAS Institute Inc., Cary, NC, USA), and the significant level was set to 0.05.

Results

Total injury (degrees 1, 2 and 3 together) value were 43.8 (24.93) and 71.81 (23.78) for PT and CT, respectively ($P = 0.0016$), and the mean difference within individuals was 26.7 (CI, 12.4-41.1), Table 4. The concrete values for total injury for each subject, considering separately the PT and CT thighs, are shown in Figure 4.

The values of each subject were, according to their treatment, 75.32 % and 67.59 % higher in CT compared with PT, for CD3+ and MPO infiltrates, respectively.

Intracellular albumin infiltrate, in absolute value, was also 32.77% higher in CT. Total inflammatory cells infiltrate were, thus, 72.07 % higher in CT vs. PT. The mean value for intracellular albumin was 1.6 (0.85) and 2.38 (0.74) for PT and CT, respectively ($P = 0.0045$). Within individuals, the mean difference found was -0.85 (CI, 0.32-1.38). MPO mean value was 1.17 (1.40) and 3.61 (4.00) for PT and CT subjects, respectively ($P = 0.001$), being the mean difference within individuals 2.56 (CI, 0.46-4.67). Similarly, intracellular CD3+ mean value was 0.57 (0.73) and 2.31 (3.11) for PT and CT, respectively ($P = 0.0313$), and the mean difference 1.88 (CI, 0.04-3.73), Table 5. When albumin infiltrates and injury degree were evaluated, CT group has more degree 1, 2 and 3 injury than PT: 11.29%, 41.18% and 54.24% respectively; conversely, PT has more degree 0 (no injury), than CT, 70.13%. The total injury was higher in CT vs PT, 39.01%, Figure 5.

The variability between individuals can be explained by some of the variables of the patient or the test. Thus, the total injury infiltrate was greater when the subject did the test at higher speeds. Subjects who did the test at more than 10km/hr, had 31% more injury, both in the PT and CT groups, while those who did the test at lower intensities <1 V-%Uan, had 21% less injury in both groups. After adjusting for these two variables the difference between the PT and the CT group was 31% (IC_{95%} = 15.4 - 43.8).

The same happened with the MPO infiltrate. The subjects who did the test at more than 10km/hr and those who did it at 1V.%Uan, had 4 times more injury. After adjusting for these variables, the CT group results showed 3.3 (IC_{95%} = 1.98 - 5.47) times more injury than the PT.

Table 4. T In: Total Injury, In 0-3: Injury degree 0-3, Dif.: Difference, SD: Standard Deviation.

	Group	Mean	Median	SD	Min.	Max.	Dif.	% Dif.	IC _{95%}		p-value
In 0	PT	50,12	56,69	28,27	0	100	-22,76	-70,13	-35,38	-10,14	0,0022
	CT	29,46	30,5	24,38	0	77					
In 1	PT	28,22	25	16,14	0	62,04	3,05	11,29	-12,18	18,28	0,662
	CT	31,81	34,5	14,33	2,85	52					
In 2	PT	15,67	11,74	15,07	0	57,78	11,78	41,18	2,08	21,48	0,0217
	CT	26,64	23	16,07	8	54,29					
In 3	PT	5,99	2,56	9,27	0	32,14	8,57	54,24	-0,2	17,34	0,0137
	CT	13,09	9	12,5	3	42,86					
T In	PT	43,8	42	24,93	0	96,67	26,7	39,01	12,36	41,05	0,0016
	CT	71,81	71	23,78	23	100					

The differences between the values of intracellular albumin and CD3+ can only be explained due to the treatment (there are no significant statistical differences in the variables of the patient or the test).

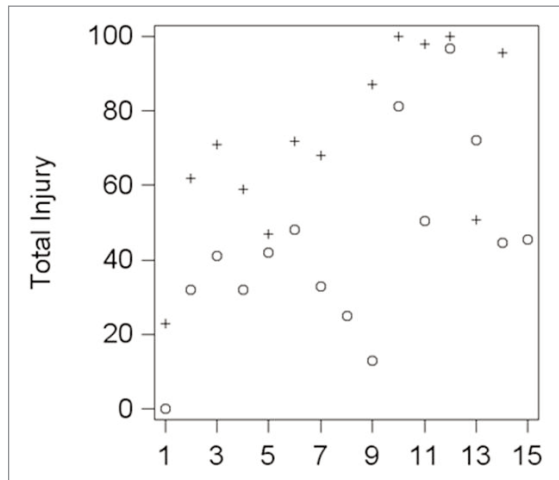


Figure 4. Scatter plots of the biopsy results for each subject. + (Control), o (Treatment).

Discussion

Many studies have evaluated the use of preventative and therapeutic measures against DOMS, but only a single reference exists – the repeated bout effect – with scientific evidence⁵⁰. Moreover, several studies involving different compression garments used in the DOMS treatment^{27,33-36} have been described, but none of them as a preventative measure.

Even being a common problem in sports medicine research, we know the sample in our study should be bigger, but is not easy to find athletes with good athletic level wanting to participate in studies, this were one of the reasons we decided to use the subject as his own control. Obviously the major part of the people was right-hand, only three subjects were left-hand in our study, and we did not find relation between the dominant hand and the amount of injury in our data analysis.

The effects of the compression tights (Colibri®, Pun-tiblond, Spain), on the biomechanics of running, high jump and tissue oscillation during intense exercise have been evaluated previously in different studies. It has been observed that the use of this garment produces a minimum decrease in hip and knee ROM without affecting the frequency and length of the

Table 5. Ic Alb: Intracellular Albumin (Absolute Value), CD3: Intra/interfibrillar CD3 infiltrate, MPO: Intra/interfibrillar Neutrophils infiltrate, TIC: Total Inflammatory cells (CD3+MPO), Dif.: Difference, SD: Standard Deviation.

	Group	Mean	Median	SD	Min.	Max.	Dif.	% Dif.	IC 95%		p-value
Ic Alb	PT	1,6	1,5	0,85	0	3	0,85	32,77	0,32	1,38	0,0045
	CT	2,38	2,5	0,74	1	3					
CD3	PT	0,57	0	0,73	0	2	1,88	75,32	0,04	3,73	0,0313
	CT	2,31	1	3,11	0	11					
MPO	PT	1,17	1	1,21	0	4	2,56	67,59	0,46	4,67	0,001
	CT	3,61	2	4	0	12					
TIC	PT	1,74	1,5	1,4	0	5	4,73	72,07	1,52	7,93	0,001
	CT	6,23	5	5,43	1	18					

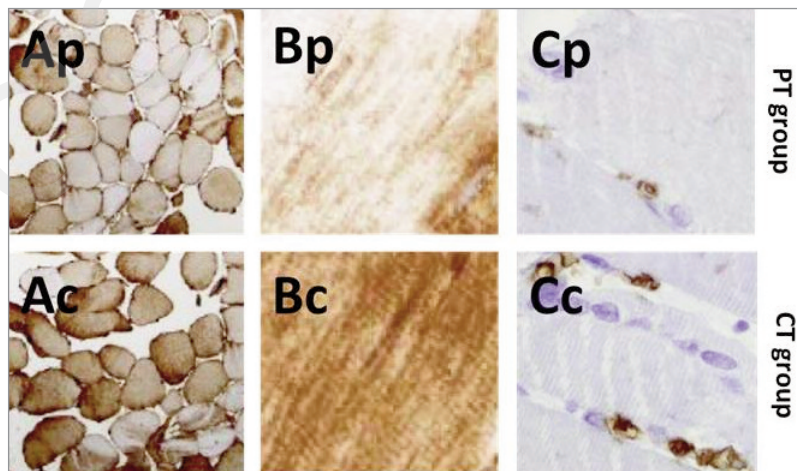


Figure 5. Biopsies. Ap: Albumin infiltrate, transversal section (PT), Ac: Albumin infiltrate, transversal section (CT). Bp: Albumin infiltrate, longitudinal section (PT), Bc: Albumin infiltrate, longitudinal section (CT), Cp: CD3 infiltrate (PT), Cc: Cd3 infiltrate (CT).

stride, without affecting the efficiency of running⁵¹; that performance in high jump is not modified⁴¹; and the amount of the inherent muscular oscillation in the race is smaller in the protected compression thigh⁵².

The reduction in muscle oscillation is related to an improvement in neurotransmission and mechanical efficiency at a molecule level^{17,20}, and also, the decrease in vibration reduces the mechanical tissue stress. And about the influence of compression garments on metabolism, although some authors have not found any differences at an energetic level¹² other groups do⁵³.

In our study we have found a reduction in the amount and severity of the histological muscle damage in the PT group, and after the statistical analysis we conclude that this reduction is because the use of the compression thighs.

The reduction in the amount and severity of muscle damage in the subjects of our study can be due to knee and hip ROM reduction, the decrease of muscular oscillation, to the direct effect of the compression on the muscle, or other factors (thermal effects, venous return, etc.), related to the use of compression garments, or a combination of several of them, further research is needed to clarify it.

DOMS is a common situation in sports practice with several signs and symptoms, but specially, with a reduction in force-generating capacity³⁷, lasting from 4 days to several weeks^{54,55}. We know that DOMS is not only a histological injury, but muscle function is considered to be a reliable and valid marker for the degree of muscle damage, and, theoretically as less histological damage, minor decrease in muscle function³⁷.

Strength is essential for athletic performance, strength disturbances are a risk factor for muscle injury⁵⁶, therefore, a garment protecting against a strength reducing injury, can be very useful.

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